Theses of the Ph.D. Dissertation

Judit Sztáray

Modeling the dissociation of protonated ions

Supervisor:

László Drahos, Ph.D., Senior Research Scientist

Institute of Structural Chemistry, Chemical Research Center, Hungarian Academy of Sciences

Chemistry Doctoral School

Head of the Doctoral School: Prof. Dr. György Inzelt

Theoretical and Physical Chemistry, Structural Chemistry Doctoral Programme
Head of the Doctoral Programme: Prof. Dr. Péter Surján

Eötvös Loránd University, Institute of Chemistry

Budapest, 2009

1. Introduction

Understanding the mechanism of ion dissociation and the major processes that influence the ion intensities observed in mass spectrometry is fundamental to advance many fields of chemistry. During my Ph.D. research, I investigated several systems to determine the fundamental properties of dissociating ions, such as the initial internal energy distribution, and the effective temperature that describe the initial state of the molecular ion. These parameters, and a carefully constructed framework to describe how they change in the mass spec experiments allows an accurate calculation of the basic features of the mass spectrum, such as ion intensities, and – in the case of metastable decays – peak widths. For this latter, my aim was also to model the kinetic energy release (KER) and kinetic isotope effect (KIE) in the dissociation of protonated dimers and clusters.

All of these carefully chosen model simulations also helped to improve our RRKM-based program package, MassKinetics so that, in the immediate future, it can be used to model a wide variety of systems with close-to-experimental accuracy.

The studied systems can be categorized into four groups: the dissociation of a) protonated alkylamines; b) protonated benzlypyridines, and protonated aromatic benzoic esters; c) protonated methanol clusters; and d) the most commonly used protonated oligopeptide, leucine enkephalin was investigated. The experimental results were taken either from previous publications or were obtained in our research group. As the principal theme of my graduate research was the modeling of mass spectra, this thesis was structured from the modeling point of view: the results were categorized into four subchapters: a) modeling ion energy distributions at different

stages of the dissociation processes of various model systems; b) modeling the kinetic energy release for protonated alkylamine and protonated methanol cluster systems; c) calculation of the kinetic isotope effect for protonated alkylamine systems; d) re-evaluation of the previously published experimental data of protonated leucine enkephalin.

2. Results and Conclusions

2. 1. Calculating Internal Energy Distributions

It is widely accepted that the internal energy and its distribution play an important role in shaping the spectrum in any mass spectrometry experiment. Therefore, as the first step in my studies, the internal energy distribution was examined using model compounds: protonated benzylpyridinium and protonated benzoic ester ions in an electrospray ionization source. These molecules are often used to determine the energy profile of a mass spectrometer because they readily yield very accurate experimental data for the modeling calculations, and for this reason they were chosen for our MassKinetics studies. In my thesis work, I have modeled the internal energy distributions of the protonated alkylamine dimers from two different experimental setups.

These ions dissociate through a loose transition state and the intensity ratio of the two possible fragment ions can be used to determine the dissociation energetics. The experimental ion intensities were used to determine the survival yield (SY), and the correlation between the critical energies and the transition states was investigated: the logarithm of the SY was found to be proportional to the logarithm of the critical energy in the case of the benzylpyridinium salts. However, the correlation was less pronounced for

the protonated benzoic ester ions. Therefore, the degree of fragmentation of the different substituted benzylpyridinium ions varies due to changes in the critical energies, whereas in the case of the protonated ester ions, it is the different transition states that lead to the different degrees of fragmentation.

The survival yields were then used to determine the corresponding characteristic temperatures. Using these, the internal energy distributions were calculated and it was found that they closely resemble thermal energy distributions. My calculations gave very similar internal energy distributions for the various substituted benzylpyridinium and benzoic ester ions, and there was no significant difference between the average internal energy distribution of the protonated benzylpyridinium ions and that of the benzoic esters, either. These results conformed the previous assumption that the electrospray ion source produces roughly the same initial internal energy distributions for systems with similar degrees of freedom.

The effect of the collisions was demonstrated by calculating the thermal energy distributions at an initial 353 K temperature plus either 20 or 40 collisions in the source and it was found that the internal energy distribution can be reproduced by a thermal distribution calculated at higher temperatures (790 K, and 1210 K, respectively). The effect of the cone voltage on the mean internal energy was also studied for both sets of the precursor ions, and an excellent linear relationship was found regardless of the ion structure.

Dissociation rate curves were calculated as a function of the internal energy and the appearance energies were determined to demonstrate the kinetic shift in the modeled experiment.

In the case of the protonated alkylamine dimers, results from two different experimental setups were taken from the literature – using a high-pressure

and a low-pressure ionization source – and MassKinetics calculations were carried out to explain the significant difference in the experimental results. In the case of the high-pressure experimental setup, the experimental results were successfully modeled assuming thermal internal energy distribution. The effective temperatures (T_{eff}) were determined from the measured ion intensity ratios (I_H/I_D = KIE) based on the kinetic method. In the case of low-pressure ionization source, the experimental and calculated T_{eff} and the ion ratios were significantly different; hence the assumption of the thermal distribution of the internal energy was refuted. The internal energy distributions were examined in detail, and, based on the experimental data, it was concluded that a) there is a high fraction of high-energy ions, leading to high fragment ion abundances, while at the same time, b) the effective temperature is low, i.e. the energy distribution are very narrow above the fragmentation threshold to account for the findings with help of the kinetic method.

It was also confirmed that the source temperature does not have a large effect on the observed T_{eff} and KIE, because the tail of the thermal distributions corresponding to the source temperature is similar above the fragmentation threshold. However, a lower source temperature results in a reduced fragment ion yield due to the smaller tail of the distribution that stretches above the fragmentation threshold.

Finally, the internal energy distribution of the fragmenting ion population under mass spectrometric conditions was compared to the internal energy distributions of ions fragmenting under thermal, equilibrium conditions (high-pressure limit). The two internal energy distributions were found to be similar at many temperatures for many of the studied systems. The roughly

similar shape of the calculated internal energy distributions serves a good qualitative support for the kinetic method that was used in this research.

2. 2. Modeling Kinetic Energy Release

Results from metastable ion measurements were used to model the kinetic energy release of protonated alkylamine dimers and protonated methanol clusters.

Statistical energy partitioning was assumed for both examined systems and the modeling results were found to be in excellent agreement with the experimental results, which show that the metastable peaks can be fitted with a single Gaussian function. The model calculations showed that KER(D) is similar to a Maxwell-Boltzmann 3-D distribution that is, the KER is due to a three dimensional translational motion, which is still a matter of debate in the literature.

Based on the results that the KERD is three dimensional, the mean kinetic energy release, <KER> was determined both from the metastable peak, and was connected to T_{eff} using the simple relationship of $\langle KER \rangle \approx 3/2\ k_B T_{eff}$. The observed agreement between the experimental kinetic energy release and $3/2\ k_B T_{eff}$ yields further proof of the three dimensional KER distribution. It is important to point out that in this calculation the T_{eff} and the KER data came from separate studies, the parameters used in the modeling were measured and calculated independently.

In the case of the protonated methanol clusters, low-temperature FAB technique was used to produce protonated methanol clusters and the KER processes were studied by recording the metastable water loss of the selected clusters. KERD curves were calculated and the distribution shapes

were examined and compared to 2-D, 3-D and 4-D Boltzmann distributions. Mean KER values, <KER>, were found to increase sharply with increasing cluster size for n = 1 – 7, and level out around octamer size. Transition state temperatures were determined using equations from three different methods: an equation describing Boltzmann distribution; an equation describing the <KER> of a thermal system; and an equation describing the FHBT. The temperatures determined by the three different ways are very similar and found to increase with cluster size: the maximum value of <KER> was about 25 meV, with a $T^{\#}$ of 210 K. These results assert that the product energy distribution can be described statistically with 3-D translation. Also, with increasing cluster size, the temperature of the transition state becomes approximately equal to the internal temperature of the cluster. This in turn applies that the internal energy of the methanol clusters is rather low, at 210K.

KER curves were also studied using MassKinetics, reproducing the 3-D curves; and the calculated mean KER values showed similar increasing tendencies with increasing cluster size.

2. 3. Modeling Kinetic Isotope Effect

The dissociation of protonated alkylamine dimers were studied by *Norrman* and *McMahon* [88], and the experimentally observed ratio of the reaction rates in non-deuterated vs. deuterated ions (k_H/k_D) was found to be larger than unity, indicating a normal secondary isotope effect. In my thesis work, this kinetic isotope effect was modeled with MassKinetics. In the case of the high-pressure ionization source, the experimental results were reproduced very well assuming thermal initial energy distribution for the molecular ions:

very good agreement between the calculated and the measured KIE values of the α-D substituted amines was found. In this case, not only the direction, but also the degree of the – very small – secondary kinetic isotope effect was successfully modeled with good accuracy. For the β-D substituted alkylamines, the experimentally observed KIEs were found to be of similar magnitude compared to the α-D substituted amines. However, the zero-point energy differences from quantum chemical methods were approximately four times smaller than for the α-D alkylamines leading to a calculated kinetic isotope effect that was much lower than the experimental values.

2. 4. The Protonated Leucine Enkephalin – Study

Leucine enkephalin was chosen for my studies because this molecule is one of the most commonly used standards in mass spectrometry. Several papers have been published reporting the Arrhenius dissociation parameters for the best studied dissociation process, the $MH^+ \rightarrow \mathbf{b_4}^+$ fragmentation reaction, however, major discrepancies can be found in the published data. The measured E_a values range from 0.94 to 1.66 eV with a standard deviation of 0.25 eV. The published pre-exponential factors also span a large range (log$A$ values from 9.1 to 15.7).

First, we found a strong correlation between the activation energy and the pre-exponential factor, connecting a high activation energy with a loose transition state (high log$A$), and a low activation energy with a tight TS. This suggests that despite the large deviation among the published results for E_a, and log$A$, the experiments might yield similar values for the Gibbs free energy of the fragmentation reaction. From the activation enthalpy, and the

pre-exponential factor – which corresponds to the activation entropy –, the $<\Delta G^{\#}>$ could be determined to be 1.34 eV with a standard deviation of only 0.03 eV (5 %). Note, however, that in this approximation, the temperature dependence of $\Delta S^{\#}$ was not taken into account.

As the next step, the published raw experimental data were used to construct an overall Arrhenius plot to investigate the temperature (or internal energy) dependence. The results show that the data are consistent, and that there are no significant deviations from a linear trend ($R^2 = 0.97$). The Arrhenius-type evaluation resulted in an activation energy of 1.14 eV and a pre-exponential factor of $10^{11.0}$ s^{-1} at the mean temperature of the experiments (489 K). The pre-exponential factor corresponds to an activation entropy of –38.1 $J \cdot mol^{-1} \cdot K^{-1}$. The effect of possible systematic errors was evaluated by including or excluding data sets from the evaluation. This shows that systematic errors are modest, and are similar to the influence of random errors. The final results (with a 95% confidence limits) are $\Delta_r H = 1.14 \pm 0.05$ eV; $\log A = 11.0 \pm 0.5$, $\Delta S^{\#} = -38.1 \pm 9.6$ $J \cdot mol^{-1} \cdot K^{-1}$, and so far represent the best available data set for the $\mathbf{b_4}^+$ dissociation channel of protonated leucine enkephalin.

This study also gives an important message for future reference: it was demonstrated that determining dissociation parameters using two or more different mass spectrometers allows one to use data from a wider temperature range and, therefore, to determine more accurate and more precise dissociation parameters from the Arrhenius plot.

3. Publications

3. 1. Articles Related to the Ph.D. Thesis

1. **J. Sztáray**, A. Memboeuf, L. Drahos, K. Vékey; Leucine Enkephalin – a mass spectrometry standard. Mass Spectrometry Reviews, 2009 accepted on 10/31/2009.
2. J. Naban-Maillet, D. Lesage, A. Bossee, Y. Gimbert, **J. Sztáray**, K. Vékey and J.C. Tabet; Internal energy distribution in electrospray ionization. Journal of Mass Spectrometry, 2005, 40(1), 1-8.
3. Á. Gömöry, P. Végh, **J. Sztáray**, L. Drahos and K. Vékey; Kinetic energy release of protonated methanol clusters using low-temperature fat-atom bombardment: experiment and theory combined. European Journal of Mass Spectrometry, 2004, 10 (2), 213-220.
4. L. Drahos, **J. Sztáray**, K. Vékey; Theoretical calculation of isotope effects, kinetic energy release and effective temperatures for alkylamines. International Journal of Mass Spectrometry, 2003, 225(3), 233-248.

3. 2. Articles Not Related to the Ph.D. Thesis

1. A.A. Tolun, H. Zhang, D. Il'yasova, **J. Sztáray**, S.P. Young, D.S. Millington; Allantoin in Human Urine Quantified by UPLC-MS/MS. Analytical Biochemistry, submitted on 10/26/2009.
2. H. Zhang, D. Il'yasova, **J. Sztáray**, S.P. Young, F. Wang, D.S. Millington; Quantification of the Oxidative Damage Biomarker 2,3-Dinor-8-Isoprostaglandin-F2α in Human Urine Using Liquid Chromatography-Tandem Mass Spectrometry. Analytical Biochemistry, submitted on 10/12/2009.

3. F. Pollreisz, Á. Gömöry**, J. Sztáray**, P. Végh, L. Drahos, A. Kiss, K. Vékey; Very high critical energy fragmentation observed in CID. International Journal of Mass Spectrometry, 243(1), 2005, 41-47
4. W.K. Lewis, B.E. Applegate, **J. Sztáray**, B. Sztáray, T. Baer, R.J. Bemish, R.E. Miller; Electron impact ionization in helium nanodroplets: Controlling fragmentation by active cooling of molecular ions. Journal of the American Chemical Society, 126 (36), 2004, 11283-11292
5. I. Bagyi, B. Balogh, A. Czajlik, O. Elias, Z. Gaspari, V. Gergely, I. Hudaky, P. Hudaky, A. Kalaszi, L. Karolyhazy, K. Keseru, R. Kiss, G. Krajsovszky, B. Lang, T. Nagy, A. Racz, A. Szentesi, T. Tabi, P. Tapolcsanyi, **J. Vaik**, J.C.P. Koo, G.A. Chass, O. Farkas, A. Perczel, P. Matyus; Generation and analysis of the conformational potential energy surfaces of N-acetyl-N-methyl-L-alanine-N'-methylamide. An exploratory ab initio study. Journal of Molecular Structure – Theochem 625(1), 2003, 121-136

3. 3. Oral presentations

1. J. Sztáray, S.P. Young, D. Il'yasova, D.S. Millington; Simultaneous detection of two biomarkers of oxidative stress using a novel UPLC-LC/ESI-MS/MS method. 2007, 25th Informal Meeting on Mass Spectrometry, Hungary
2. J. Sztáray, S.P. Young, D. Il'yasova, D.S. Millington; Simultaneous detection of two biomarkers of oxidative stress using a novel UPLC-LC/ESI-MS/MS method. 2007, 55th American Society of Mass Spectrometry Conference, Indianapolis, UDA

3. J. Vaik, L. Drahos, K. Vékey; Understanding Structure and Reactivity of Ion-Dipole Clusters. 2000, 19th Informal Meeting on Mass Spectrometry, Hungary

3. 4. Poster presentations

1. J. Sztáray, K. Vékey, L. Drahos; Modeling Fragmentation in Electrospray. 2005, 24th Informal Meeting on Mass Spectrometry, Italy
2. J. Sztáray, L. Drahos, Á. Gömöry, P. Végh, K. Vékey; Theoretical Calculation of Isotope effects, Kinetic Energy Release and Effective Temperatures of Selected Systems. 2003, Gordon Research Conference on Gaseous Ions, Ventura, CA
3. J. Sztáray, B. Sztáray, R.E. Miller, T. Baer; Dissociation Dynamics of Triphenylmethanol Studied by Threshold Photoelectron Photoion Coincidence Spectroscopy. 2003, Gordon Research Conference on Gaseous Ions, Ventura, CA
4. J. Vaik, J. Zádor, R. Szalay, D. Knausz; Silyl-substituted thiocarbamates. 1999, XIII. FECHEM Conference on Organometallic Chemistry, Lisboa, Portugal